Functional Expression of Cytochrome P450 Enzymes in Human Hepatoma HepaRG Cell Line

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The spectrum of major drug-metabolising cytochrome P450 (CYP) enzyme activities and the response to prototypical inducer and CYP-selective chemical inhibitors in human hepatocellular carcinoma derived cell line, hepaRG, was determined using an N-in-one assay. HepaRG cells were found to express high functional levels of the major CYPs involved in xenobiotic metabolism, which were selectively inhibited and induced by prototypical CYP-selective inhibitors and inducers.

BACKGROUND Hepatocyte cell lines, mainly originated from tumours, have extended proliferative capacity but they lack a variable and substantial set of liver-specific functions, making them unsuitable as representative of *in vivo* hepatocytes. In particular, CYP expression is usually very low or altogether undetectable in human hepatoma cells. Recently, a new human hepatoma cell line derived from an hepatocellular carcinoma, named HepaRG, that exhibits extensive differentiation after 2 weeks at confluency, was established (1). Later studies demonstrated that, in conditions in which cells attain a differentiated hepatocyte-like morphology, they retain a unique set of drug metabolizing enzymes, including some CYP-associated

MATERIALS & METHODS The incubations were performed either in the presence of the differentiation medium, which contains DMSO (DIFF), or after the switch to the maintenance medium, Williams E (WE) (1,2). Primary hepatocytes (Hepat) were cultured in Williams E medium. We used an N-in-one assay of ten CYP-selective probe substrates by which all major CYP activities can be followed simultaneously as a function of time up to 48 h (3,4). After addition of the N-in-one MIX, in two concentrations (1xMIX and 10xMIX), incubation periods of 0, 2, 4, 8, 12, 24 and 48 h hours were carried out. For induction studies, cells were treated with 1.5 mM phenobarbital (or solvent vehicle) and for inhibition studies, the inhibitors were

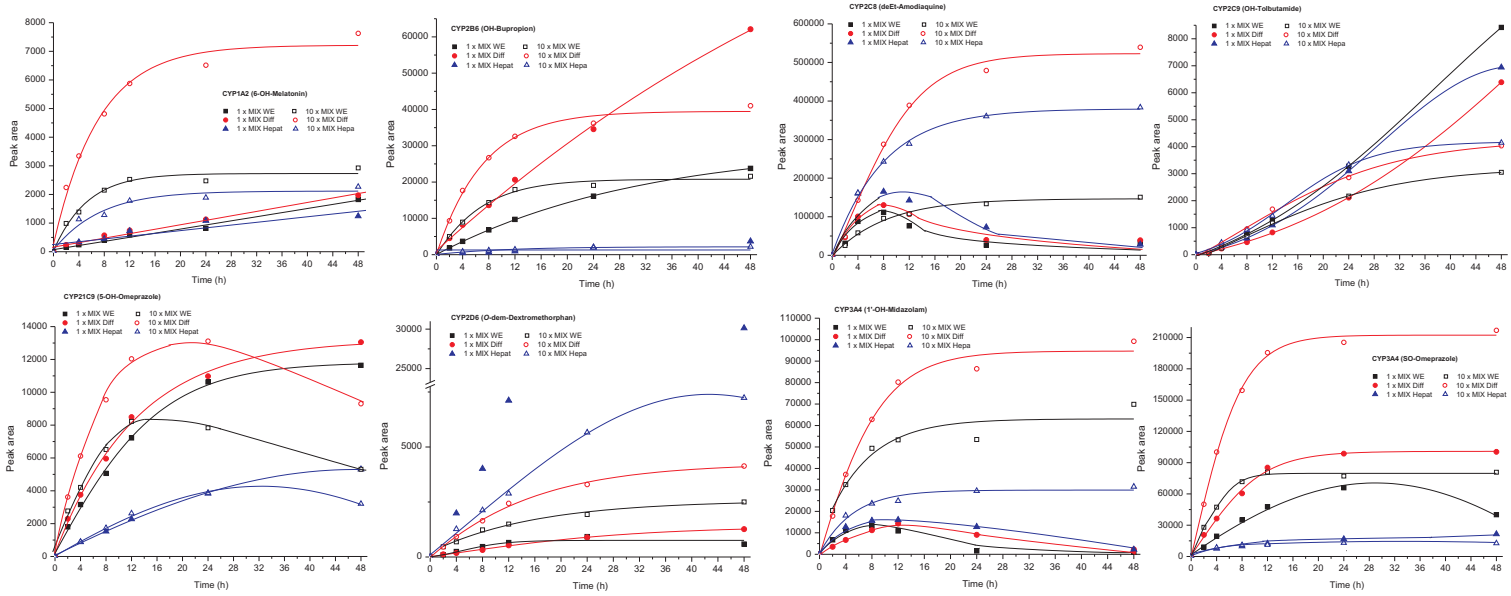

Figure 1. Functional expression of CYP-associated activities in HepaRG cells and in primary human hepatocytes.Differentiation medium (DIFF) maintenance medium (WE), primary hepatocytes (Hepat)

CYP	Diagnostic inhibitor	HepaRG	Literature (3,4)
1A2	Furafylline	< 0.1	< 0.1
2A6	Tranylcypromine	< 0.1	0.4
2B6	Ticlopidine	< 0.1	0.35
2C8	Montelukast	2.6	0.4
2C9	Sulphaphenazole	< 0.1	0.35
2C19	Fluconazole	42.5	23.8
2D6	Quinidine	< 0.1	< 0.1
3A4	Ketoconazole	< 0.1	0.06

Table 1. IC_{50} values (μM) of diagnostic inhibitors added simultaneously with the n-in-one cocktail and incubated for 24 hours.

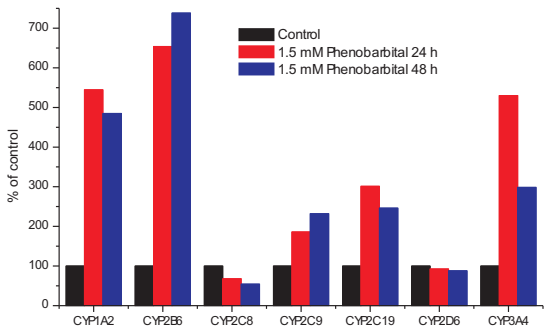

Figure 2. Induction of CYP-associated activities with phenobarbital

CONCLUSIONS HepaRG cells express high functional levels of the major CYPs involved in xenobiotic metabolism, which were selectively inhibited and induced by prototypical CYP-selective inhibitors and inducers.

REFERENCES (1) Aninat et al. Drug Metab Dispos. 2006, 34:75-83; (2) Gripon et al. Proc Natl Acad Sci U S A. 2002, 99:15655-60; (3) Turpeinen et al. Eur J Pharm Sci. 2005, 24:123-32; (4) Turpeinen et al. Eur J Pharm Sci. 2006, 29:130-8.

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